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Investigating the role of CheA-3 in *Desulfovibrio vulgaris* Hildenborough

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ENIGMA — Ecosystems and Networks Integrated with Genes and Molecular Assemblies

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Abstract:

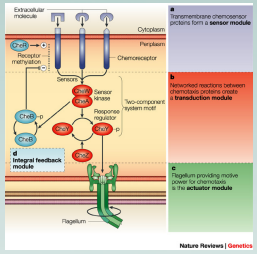
Multiple sets of chemotaxis genes including three *cheA* homologs were identified in the genome sequence of the anaerobic bacterium *Desulfovibrio vulgaris* Hildenborough. Each CheA is a histidine kinase (HK) and part of a two component signal transduction system. Knock out mutants in the three *cheA* genes were created using single cross-over homologous recombination insertion. We studied the phenotypes of the *cheA* mutants in detail and discovered that Δ*cheA-3* has a non swarming/swimming phenotype both in the soft agar plates and Palleroni chamber assays. CheA-3 shows similarity to the *Shewanella oneidensis* CheA-3 and the *Vibrio cholerae* CheA-2 that are responsible for chemotaxis in the respective organisms. We did not find any morphological or structural differences between the three Δ*cheA* mutants and the wild type cells in electron microscopy. Our results from these studies are presented.

Introduction:

Objective: To gain a detailed understanding of the *D. vulgaris* Hildenborough two-component signal transduction systems responsible for chemotaxis.

D. vulgaris Hildenborough has 3 chemotaxis sensor histidine kinases named CheA-1, CheA-2 and CheA-3. They may be involved in separate chemotaxis functions (e.g. taxis towards electron acceptor.

Bacterial Chemotaxis System

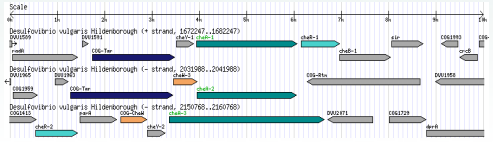


McAdams, H.H. et al. *Nature Reviews Genetics* 5, 169-178 (March 2004)

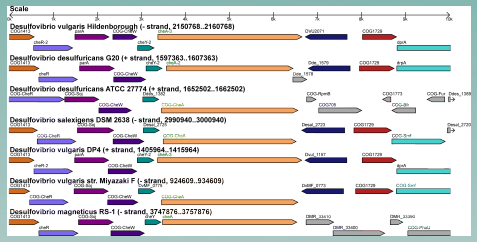
D. vulgaris Hildenborough and Two-Component Systems (TCS):

- Anaerobic Sulfate Reducing Bacteria (SRB).
- Found in heavy metal and nuclear waste site.
- Genome was sequenced in 2003.
- **A large number of TCS were identified in *D. vulgaris* including 64 putative sensor histidine kinases and 72 putative response regulators.**
- TCS in bacteria are known to regulate key environmental and stress responses. However, functions of most of the *D. vulgaris* TCS are unknown so far.

Sensor histidine kinases in chemotaxis gene containing operons of *D. vulgaris* Hildenborough

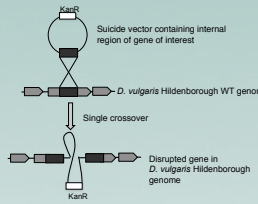


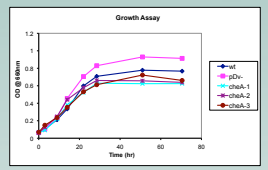
The operon containing histidine kinase *cheA-3* is the most conserved.



Methods:

HK knock-out mutants using Single cross-over homologous recombination





In liquid LS4D medium all strain show equivalent growth

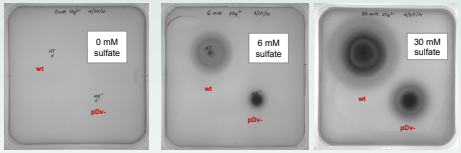
Characterization of the *cheA* knock-out mutants

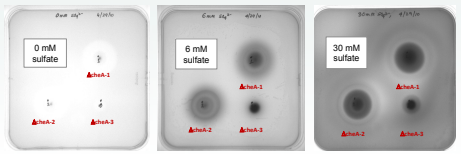
- ❑ Swarm/swim plate assay
- ❑ Palleroni chamber assay
- ❑ Electron microscopy imaging

Results:

❑ Swarm/swim plate assay:

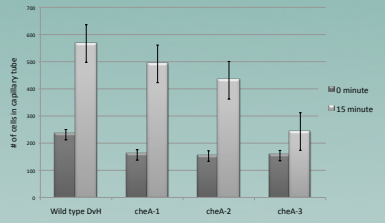
Soft agar plates: Lactate sulfate media with 0.4% agar, 60mM lactate and varying sulfate concentration. 30mM sulfate would be considered non-limiting for *D. vulgaris*





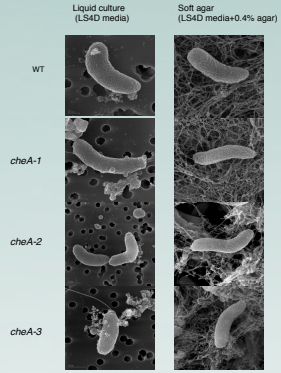
❑ Palleroni chamber assay:

Motility assay in a lucite plate containing four chambers. Chamber were filled with bacterial suspension and thin capillaries (both ends open) were filled with chemo-attractant and placed in the pre-filled chambers. Capillaries were picked up after desired time and number of cells in the capillaries were counted using FACS (Fluorescence activated cell sorter).



❑ Electron Microscopy (SEM images):

Cells were grown to mid-log and fixed with 2% gluteraldehyde before imaging.



Summary:

- Three *cheA* gene knock-out mutants have been created using single cross-over homologous recombination insertion.
- We have investigated the role of all three CheAs in motility of *D. vulgaris* Hildenborough in chemotaxis assays
- Δ*cheA-1* and Δ*cheA-2* mutants have similar motility as the wild type in the swarm/swim plate assays.
- Δ*cheA-3* mutant shows almost complete loss of motility in the swarm/swim plates. This suggests that CheA-3 may be essential for chemotaxis in *D. vulgaris* Hildenborough.
- No noticeable morphological or structural differences between the wild-type and the mutant cells were seen in the scanning electron microscopy.

ACKNOWLEDGEMENTS

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